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Expression of notch receptors and their ligands in the adult human articular cartilage and colony forming cells

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Purpose: Notch signaling plays a role in cell fate determination in many different tissues in multicellular organisms, for example, in nervous system, vascular system, hematopoietic system, somites, muscle, skin and pancreas. Therefore, we aimed to examine the expression of notch receptors and their ligands in adult human articular cartilage and colony cells by the immunohistochemical techniques.

Methods and Materials: In this study we used human articular cartilage from 15 individuals (n=15) ranging from 20 to 40 years. Biopsies were obtained after consent forms were taken from patients undergoing arthroscopic surgery. Explants were taken from the distal portion of the femur and were approximately 0.3 cm² in size. Permission for the removal of biopsies was granted by Akdeniz University Medical Faculty Ethic Committee. The samples were cryosectioned and immunostained for Notch1-4 and their ligands Delta, Jagged1 and 2 or enzymatically digested and the resulting cells cultured and plated on fibronectin coated dishes. Chondrocytes were cultured until colonies of >32 cells were present. After then, expression of Notch and its ligands were detected in vitro using immunohistochemistry.

Results: Expression of Notch-1 and Delta were observed more densely than other Notch receptors (Notch-2, Notch-3 and Notch-4) and their ligands (Jagged-1 and Jagged-2) in articular cartilage sections. Colony forming cells more densely expressed Notch-1, Delta-1 and Jagged-1 than other Notches and their ligands in the monolayer culture.

Conclusions: We suggest that adult human articular cartilage may have progenitor-like cells. Nevertheless, more studies need to be carried out regarding the progenitor/stem cells in healthy normal human articular cartilage tissue.

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The Expression Of Notch-1, Delta-1, Cd105 And Cd166 In Femur Head Articular Cartilage Of High Dosage Corticosteroid Treated Rats

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Purpose: Although it is known that high dosage corticosteroid treatment involves some degenerative changes in articular cartilage it is mysterious that how the expression of Notch-1-cell fate determinative membran receptor-, its ligand Delta and stem cell markers CD105 and CD 166 change and this should be clarified. So we aimed to study the effects of corticosteroid treatment on the expression of Notch-1, Delta, CD105 and CD166 on rat femur head articular cartilage.

Methods and Materials: In the study 24 female Wistar rats were used. Rats were separated into 2 groups randomly. 1. group rats (n=8) were treated only with isotonic salt solution and those were the control group. 2. group rats (n=8) were treated with 10mg/kg corticosteroid intramuscularly once a week during 2 months and those were the corticosteroid treated group. After treatment had finished rats were sacrificed and femur heads were obtained. Immunohistochemistry was applied to examine the expressions of Notch-1, Delta, CD105 and CD166.

Results: In both control and corticosteroid treated groups all markers decreased from surface through deeper zones. However expression intensity was lower in corticosteroid treated group. This difference was statistically significant for Notch-1, Delta and CD166 (p<0.001) but not for CD105 (p>0.001).

Conclusions: Corticosteroid treatment may cause to decrease the expressions of cell fate related markers and affect the normal structure of chondrocytes.

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Expression of Fas and Fas Ligand (FasL) in femur head articular cartilage of high dosage corticosteroid treated rats

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Purpose: Corticosteroids are used as an inflammatory agent for treatment of some diseases. It is known that long term corticosteroid treatment cause the necrosis of bone cells and they have apoptotic effect on osteoblast and osteocytes. Although degenerative effects are known it is mysterious that how apoptotic pathways proceed. So we aimed to investigate the expression and zonal distribution of Fas and FasL that takes part in apoptotic pathway in femur head articular cartilage of corticosteroid treated rats.

Methods and Materials: In the study 16 female Wistar rats were used. Rats were separated into 2 groups randomly. Control group rats (n=8) were treated only with isotonic salt solution. Corticosteroid group rats (n=8) were treated with 10mg/kg corticosteroid intramuscularly once a week during 2 months. After treatment had finished rats were sacrificed and femur heads were obtained. Immunohistochemistry was applied to examine the expressions Fas and FasL.

Results: In both control and corticosteroid treated groups Fas and FasL expressing cells decreased from surface through deeper zones in articular cartilage. According to our H-SCORE results; Fas expression was more in control group and FasL expression was more in corticosteroid treated group. But the difference wasn't statistically significant (p>0.05).

Conclusions: Corticosteroid treatment may show its apoptotic effect via Fas and FasL pathway. However to be able to get accurate results it is indispensable to do more detailed and molecular studies.

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Analysis of human articular chondrocytes and adipose derived stem cells in co-culture under three dimensional conditions

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Purpose: Autologous chondrocyte implantation (ACI) is a powerful technique to treat cartilage defects. In order to keep donor site morbidity low, only small cartilage biopsies are used for primary chondrocyte isolation. Since adipose tissue derived stem cells (ASC) can be easily isolated and also show the ability to differentiate into the chondrogenic lineage, we hypothesise that a partial substitution of chondrocytes with ASC might be a possibility to reduce the number of needed chondrocytes in ACI.

Methods and Materials: Chondrocytes were obtained from human donors undergoing an arthroscopic procedure. Briefly, cells were isolated by collagenase digestion and expanded up to passage 2. ASC were isolated from liposuction material and passage 4 cells were used for the study. Different ratios of chondrocytes to ASC were seeded either in fibrin glue (Tisseel) or collagen (Tissue Fleece®). Histology stainings were performed after 4 weeks of static cultivation in chondrogenic differentiation medium. RNA for quantitative RT-PCR was isolated after 2 weeks.

Results: Van Giessen staining showed matrix production of cells seeded to both, fibrin glue and Tissue Fleece®. Histology stainings also demonstrated remarkable differences in matrix density dependent on the ratio of chondrocytes to ASC. Expression of different matrix protein encoding genes such as collagen I, collagen II, aggrecan and versican could be shown by RT-PCR.

Conclusions: The results of this study might suggest that the use of coculture systems in combination with suitable scaffolds could be a useful approach for cartilage tissue engineering.